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14. ABSTRACT

Ovarian epithelial tumors are highly diverse and the exact tissue of origin is still unclear. Although the ovarian surface epithelial cells (OSEs) are believed to be the originating site, more recent evidence suggests that the ovarian tumors could instead arise directly from extraovarian tissues that are embryologically derived from the müllerian ducts. Using genetically engineered mice (Cre-loxP) with conditional mutations in the Kras and PI3K/Pten pathways (KrasPten mice), we demonstrated in year one that activation of these oncogenic pathways in the peritoneal cavity, may lead to ovarian tumors that are histomorphologically identical to those arising in mice with Kras and PI3K pathways activated in the OSE only. Further validation of these novel findings via expression profiling is currently in progress. If confirmed, our work may lead to identification of a new paradigm in ovarian tumorigenesis, one that may not require the involvement of the OSE.

We also identified for the first time that activation of the Kras and PI3K pathways in the uterine lining leads to endometrial hyperplasia, a premalignant condition, predisposing to endometrial adenocarcinoma. This animal model would provide unparalleled opportunities for studies on premalignant and malignant lesions of the female genital tract, with secondary ovarian involvement.

Table of Contents

	<u>Page</u>
Introduction.....	5
Body.....	5
Key Research Accomplishments.....	14
Reportable Outcomes.....	15
Conclusion.....	15
References.....	16
Appendices.....	17

INTRODUCTION:

Ovarian epithelial tumors are highly diverse and the exact tissue of origin is still unclear. Until recently, all five histological subtypes of ovarian epithelial tumors (serous, endometrioid, mucinous, clear cell and transitional adenocarcinomas) were believed to arise in the coelomic epithelium that covers the ovarian surface epithelium (OSE) which then undergoes metaplasia and changes to müllerian -like epithelium before malignant transformation. More recently, however, it has been suggested that they could instead arise directly from extraovarian tissues that are embryologically derived from the müllerian ducts. Although scientific evidence in support of both theories exists, further studies on disease pathogenesis are needed.

Our studies use previously described, genetically engineered mice (Cre-loxP) that carry the lox-Stop-loxP-KrasG12D oncoallele and a floxed region within region encoding for the phosphatase domain of the Pten gene (KrasPten mice- (1)). These silent mutations are present in all cells/tissues of the mouse body and are activated when the cells from a particular anatomic location becomes infected by a Cre recombinase encoding adenovirus (AdCre). It has been previously shown that when AdCre is injected in the ovarian bursa, the Kras and PI3K/Pten pathways are both triggered in the OSE and the mice develop ovarian tumors with endometrioid histology (1).

We postulate that similarly to intrabursal injections, AdCre injection along various other sites of the genital tract of KrasPten mice will allow us to study *in vivo* tumor initiation and progression and to identify important disease pathogenesis mechanisms in ovarian tumors and other cancers of the genital tract. Furthermore, these studies may provide unique models for identification of disease biomarkers and for in vivo testing of novel immune therapies. Our studies focus on three

Specific Aims:

Aim 1. To investigate the müllerian tract versus the OSE as the potential originating sites for ovarian epithelial tumors in KrasPten mice.

Aim 2. To profile disease heterogeneity and to identify immune biomarkers of natural and vaccine-induced immune responses in mice with either endometriosis, ovarian cancer or endometriosis progressing to ovarian cancer.

Aim 3. To validate in human specimens the disease biomarkers identified (in aim 2) in mice with endometriosis and ovarian tumors.

BODY

We present below our progress (year 1) according to the tasks and milestones described in the original application.

Aim 1 (Months 1-18). To investigate the Müllerian tract versus the OSE as the potential originating sites for ovarian epithelial tumors in KrasPten mice.

Task 1 (Months 0-2). To secure IACUC approval.

The work was started on a pre-existent, approved protocol. In April 2011, the PI has applied for and was granted IACUC approval for additional 3 years (according to the University of Pittsburgh policy regarding new applications, needed every 3 years- approval letter attached as appendix). All the experiments outlined here are performed as described in the original grant submission and as stated in the IACUC protocol.

Task 2 (Months 0-6). To set up large animal breeding protocols and implement genetic screening of littermates.

Breeding protocols have been started and we are continuously screening large number of litters (more than 240 mice/year), as originally proposed. The tail DNA extraction and PCR protocols for genotyping have been performed according to protocols described by us and others (1, 2). The breeding typically pairs consist of one MUC1^{+/-}Pten^{loxP/loxP} female mouse crossed with one lox-Stop-loxP-KrasG12D (LSL-KrasG12D, or briefly KrasPten) male mouse.

To identify the experimental and control mice, we genotype female mice from all litters using primers specific for the lox-Stop-loxP-KrasG12D (LSL-KrasG12D) and MUC1 transgenes respectively (Fig. 1). Among the female mice of each litter, one in 4 is expected to be triple Tg (MUC1KrasPten). Expected frequency of females that are double Tg (KrasPten) is 1 in 2. The KrasPten and the MUC1KrasPten female mice were used for the experiments below.

For selection of breeders, males were also occasionally screened.

Separate lines are propagated in order to maintain the single transgenic (Tg) breeding lines (MUC1^{+/-} and Kras^{G12D/+}, respectively) on a heterozygous background. Single transgenic and/or wild type mice are also used as controls, as needed.

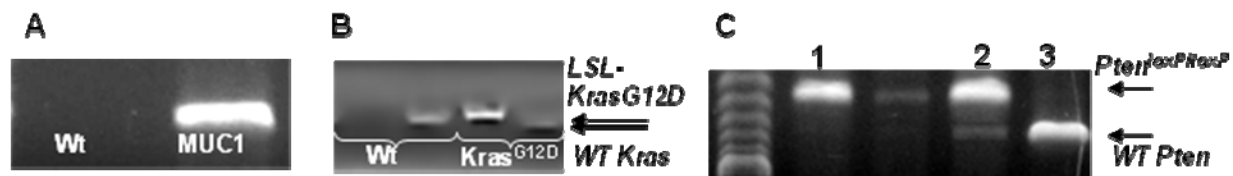


Fig. 1. Example of PCR results. Tail DNA was extracted from 4 week old pups and amplified with primers specific for either human MUC1 (A), loxP-Stop-loxP-KrasG12D (LSL-KrasG12D) (B) and Pten^{loxP/loxP} (C). Mice are then tagged according to the presence of the human transgene and of the loxP sites in the Kras and Pten genes (indicated by arrows in B and C, respectively). Breeders need to be homozygous for the presence of the loxP sequence at the Pten locus (lane 1, in panel C).

Task 3 (Months 6-9). To complete survival surgery and AdCre injection via 4 routes: intrabursal, intraductal, intrauterine and intraperitoneal.

We have completed survival surgery and AdCre injections via the intra-bursal, intra-ductal, intra-uterine and intra-peritoneal routes (Fig. 2) in a total of 40 mice.

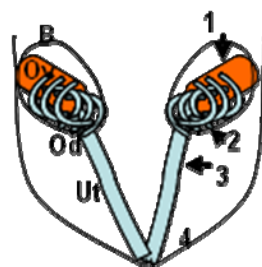


Fig. 2. Diagram of injection routes. Four-to six month old female mice were injected with adenovirus encoding for Cre recombinase via four routes: (1) under the ovarian bursa; (2) in the oviduct; (3) in the uterine horn and (4) in the peritoneal cavity. In mice, the ovaries (Ov) are surrounded by a bursa (B). The oviduct (Od) connects the ovaries to the uterine horns (Ut).

- **Intrabursal (IB) injections**

We have so far performed AdCre injections (2.5×10^7 plaque forming units, pfu) under the bursa of double and triple Tg mice (KrasPten and MUC1KrasPten, respectively, Table 1).

Our results show that the IB injections lead to primary ovarian tumors with endometrioid histology in both triple and double Tg mice (Fig. 3). The tumors develop at the primary site and at late stages, they spread throughout the peritoneal cavity and on the diaphragm, mirroring the human disease. However, the MUC1 + tumors from triple Tg MUC1KrasPten mice are more metastatic and trigger increased loco-regional immune suppression. Our findings on MUC1-induced differences in tumor biology and loco-regional immunity were separately studied (Budiu et al, manuscript under review).

The IB-induced ovarian tumors were used as positive controls for experiments in aim 1.

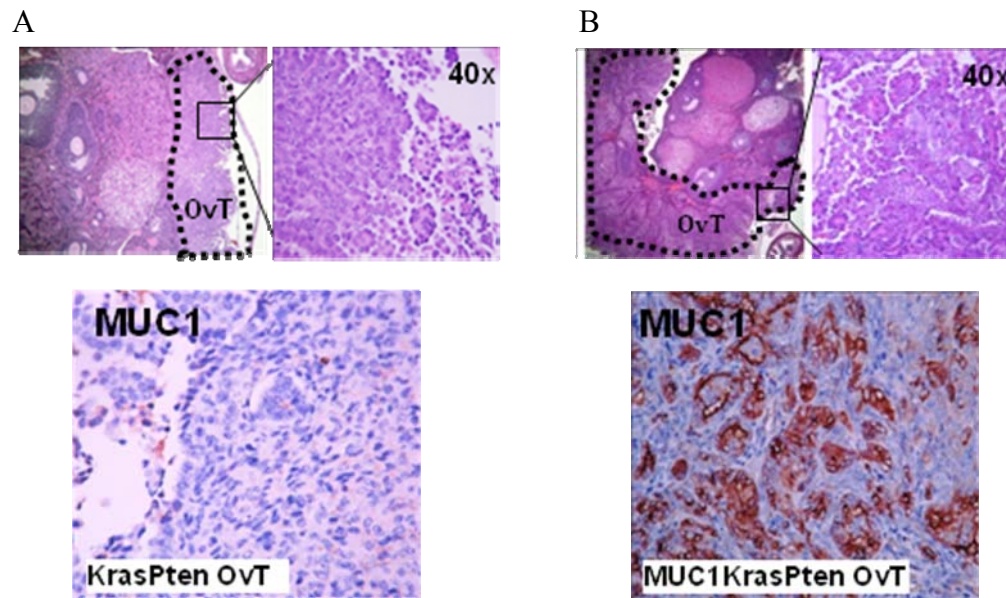


Fig. 3. The ovarian tumors (OvT) in the double (KrasPten) and triple (MUC1KrasPten) mice have endometrioid histology (H&E staining, upper panels in A and B). Human MUC1 is present in triple Tg tumors only (B, lower panel) as detected by immunohistochemistry using a MUC1-specific monoclonal antibody (clone HMPV). As expected no MUC1 staining is detected in KrasPten tumors (A, lower panel).

- Intraductal (ID) injections: As with the IB injections, the ID injections were performed under the microscope during survival surgery, as originally proposed. We have initially attempted injections in n=4 mice, using the same needles used for IB injections (26G). However, due to the extremely small diameter of the oviduct, we experienced difficulties targeting the lumen of the duct, as the needle often pierced through it. To address this, we had contacted Hamilton company and ordered custom made needles (34G) and 5 μ L syringes. Also, to seal off communication between the oviduct and the ovary (distally) and uterine horn (proximally) we had acquired microsurgical staples and staple applicator. Using these newly acquired tools we plan to complete soon the in vivo ID AdCre administration in n=10 mice, as proposed.

- Intrauterine (IU) injections: We have completed injections in a total of n= 8 mice (three KrasPtenP, three MUC1KrasPten, and two Pten). Experiments are pending in five additional mice. During this surgical procedure, the left uterine horn is exposed and 2×10^7 AdCre pfu are injected unilaterally, with the needle pointing away from the oviduct and ovary. Results from these experiments are discussed below.

- Intraperitoneal (IP) injections: We injected IP a total of n = 12 mice (three KrasPten, four MUC1KrasPten, five MUC1Pten) with AdCre at 5×10^8 pfu.

Control (healthy, uninjected) mice were also included, as per the original protocol.

Results from these experiments are discussed below.

Task 4 (Months 6-15).

- Disease monitoring, necropsy, sample acquisition and analysis.

After injection, all animals were monitored for disease development. When moribund (unable to reach for food and water, hunched appearance etc), the mice were sacrificed as described in the proposal and according to the IACUC protocol. For mice with no signs of disease, the experiment was terminated 8 weeks after AdCre injection.

At necropsy, the following biological specimens were collected from all mice:

- ✓ Ascites fluid (when present). Supernatant and cells were separately cryopreserved. When ascites was not present, we performed peritoneal lavage (IP wash);
- ✓ Blood was collected via cardiac puncture and used for serum collection;
- ✓ The entire genital tract (ovaries + oviduct+ uterus) was dissected and used for formalin fixation followed by paraffin embedding and histology [(hematoxyllin/eosin (H&E), immunohistochemistry, (IHC)];
- ✓ Spleen, lymph nodes (inguinal, para-aortic) were processed into single cell suspensions and cryopreserved;
- ✓ When present, metastatic tumors were also separately collected and processed for histology.

- Pathology analysis of affected organs: immunohistochemistry (IHC), laser capture microdissection (LCM), RNA extraction, PCR arrays (months 6-15).

Pathology results (IHC, LCM)

To identify any histological abnormalities of the genital tract, all paraffin embedded blocks (ovaries+oviduct+uterus) were step-sectioned and stained with H&E.

For the microscopical examination of all lesions we have consulted with Esther Elishaev, MD, a pathologist with expertise in gynecologic diseases.

Summary of findings:

1. Intraperitoneal AdCre injection triggers ovarian tumors that are histomorphologically identical with the primary ovarian tumors arising after IB AdCre (both with endometrioid histology

(Figures 4 and 5). However, the tumor penetrance is not 100% (as seen post- IB administration) and when present, the tumors are much smaller, the ovaries often appearing macroscopically normal (Fig. 4).

Intrabursal (IB) AdCre
2469



Intraperitoneal (IP) AdCre
2692



Fig. 4. Macroscopic appearance of the female genital tract in AdCre injected mice. Four-to-six month old female KrasPten mice were injected with Cre-encoding adenovirus (AdCre) either under the ovarian bursa (IB, left) or intraperitoneally (IP, right). The mice were sacrificed when moribund and the organs collected, fixed and paraffin-embedded for histology.

An example of 3 different KrasPten mice (#2688, #2717 and #2371) injected IP is shown in Fig. 5. The right panels show one normal, healthy KrasPten negative control (non- injected) and one KrasPten mouse with ovarian tumor injected IB (positive control). A summary of tumor characteristics in KrasPten mice injected IP versus IB is presented in Table 1.

Table 1. Characteristics of tumors triggered via IB and IP AdCre

	Intrabursal AdCre N = 34 n(KP) = 21; n(MKP) = 13	Intraperitoneal AdCre N =12 n(KP) = 3; n(MKP) = 4; n(MP) = 5
Disease penetrance	100%	50%
Time to progression	6-8 Weeks	Variable
Ascites	Frequently	Frequently
Sudden death	No	Yes, need for close monitoring
OvT Histology	Endometrioid	Endometrioid
Endometrium	No changes	Cystically dilated glands Complex glandular hyperplasia Apoptotic debris, invasion of stroma

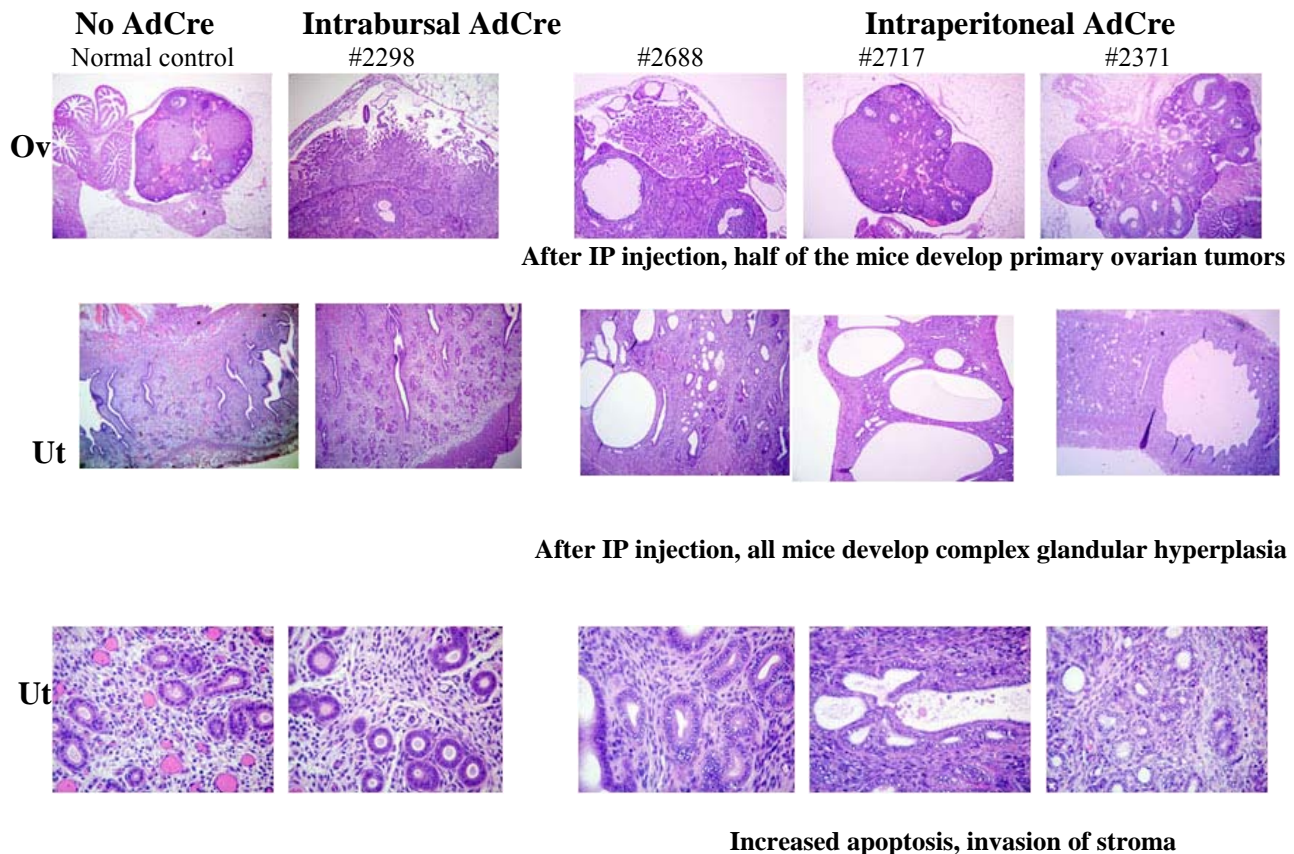


Fig. 5. Histomorphologic assessment of lesions of the ovaries (Ov, upper panels) and uterus (Ut, middle and lower panels) from mice injected via the intrabursal (#2298) or the intraperitoneal (#2688, #2717, #2371) route. Healthy, non-injected mice served as control (left column). The H&E staining shows that when present, the tumors have endometrioid histology in both IB and IP injected mice (#2298 and #2688, respectively). Although some of the IP injected mice do not have ovarian tumors (#2717 and #2371), all mice develop complex glandular hyperplasia (middle panels, IP injected mice), in contrast to IB injected mice that have normal endometrial histology. The endometrium of IP injected mice also shows increased apoptosis and invasion of stroma (lower panels, IP injected mice). A summary of observed changes is shown in Table 1.

Our results demonstrate that when present, the tumors arising in the ovaries of IP injected mice are histomorphologically identical with those in IB injected mice. Furthermore, these findings demonstrate for the first time that ovarian tumorigenesis can be triggered in the KrasPten mice via intraperitoneal injection of AdCre.

2. Intraperitoneal but not intrabursal AdCre administration also leads to abnormal endometrial histology (Fig.5). The IP injected mice have cystically dilated glands and morphological changes consistent with complex glandular hyperplasia. The presence of nuclear atypia is occasionally noted. These results suggest that the ovarian tumorigenesis post-IP injection develops in the context of a more complex pathology, involving adjacent organs of the genital tract.

3. Intraperitoneal injected AdCre does not seem to directly infect the OSE.

Given the highly similar histology in tumors triggered after IP and IB AdCre, we asked next whether the phenotype observed may be due to a systemic infectivity of AdCre, resulting in the adenovirus reaching the OSE after IP injection. Mouse ovaries are enclosed in the bursa, which we postulate is protective against the OSE from being directly infected by the IP injected adenovirus. To test this, we injected the mice IP with β -galactosidase encoding adenovirus (AdLacZ). If the virus would directly infect the OSE after IP injection, the infected cells could be detected by exposure of the ovaries to b-gal substrate which renders the infected (β -gal expressing) cells blue.

Our results show that IP injection does not trigger blue coloring of either the OSE or the endometrial lining (Fig. 6), suggesting that the ovarian tumors may have been triggered by mechanisms other than direct OSE involvement. To further confirm these findings, we have initiated DNA and RNA expression profiling of primary tumors triggered IP versus those triggered IB, which will potentially lead to the identification of site-specific pathways, able to distinguish the pathogenic mechanisms.

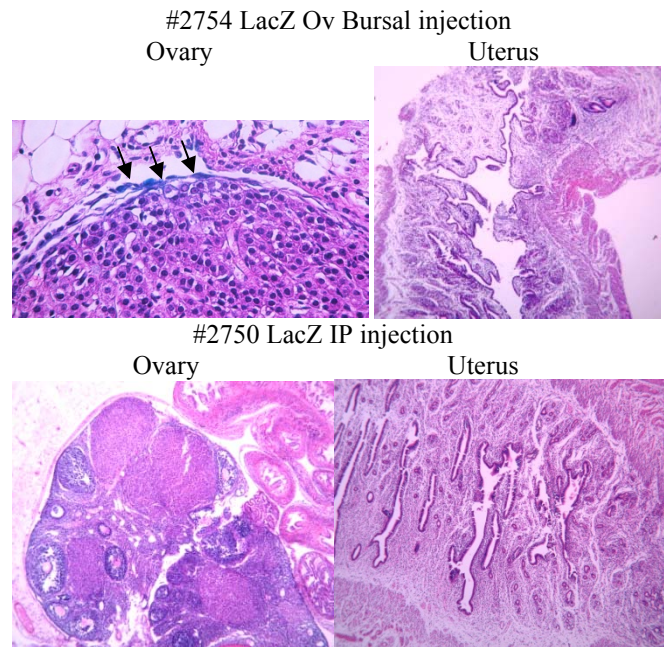


Fig. 6. Similarly to AdCre injections, we performed injection of AdLacZ either IB (upper panels) or IP (lower panels). The mice were sacrificed at either 3, 5 or 7 days after injection and organs of the genital tract were exposed to b-gal substrate. Results from day 5 experiments are shown. After macroscopic examination for blue staining, the organs were fixed, paraffin embedded and step-sectioned for HE staining. As expected, detected blue OSEs when the adenovirus was injected IB (upper left panel, arrows). No blue cells were detected in the uterine lining post- IB injections. Similarly, no blue cells could be detected post-IP injections, on either the ovarian surface or uterine lining.

4. Intrauterine AdCre administration leads to glandular hyperplasia and endometrial tumors with squamous differentiation.

These experiments allowed us to identify for the first time that direct activation of the Kras/Pten pathways in the endometrial epithelium triggers glandular hyperplasia, a pre-malignant condition. Furthermore, in a subset of the mice with IU AdCre injections, large primary endometrial tumors were also noted (Fig. 7). The tumors show squamous differentiation, consistent with the histopathological diagnosis of endometrioid endometrial tumors. Although glandular hyperplasia was found in all IU injected mice (100% penetrance), only 2 of the six mice (one double and one triple Tg) developed tumors. None of the single Tg Pten mice developed tumors.

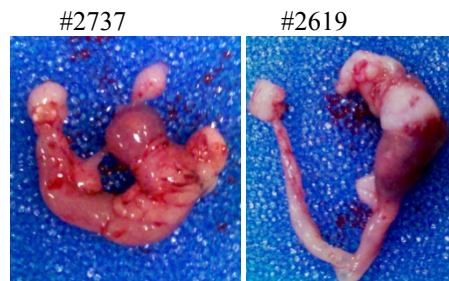


Fig. 7. Macroscopic appearance of the genital tract of two female KrasPten mice injected IU with AdCre. The uterine tumors are clearly visible in both mice and occurred in the injected uterine horns. The tumor in mouse #2619 has expanded to the ipsilateral ovary (secondary ovarian tumor).

The image in Fig.8 shows one example (#2619) of a large, primary endometrial tumor that has developed secondary ovarian tumor.

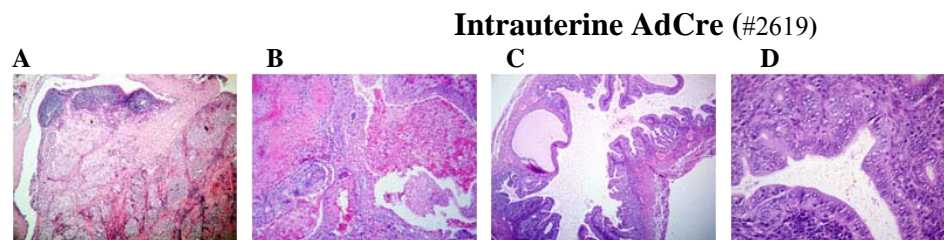


Fig. 8. Histomorphological changes of lesions throughout the genital tract of one mouse (#2619) injected IU with AdCre. (A) The tumor has spread to the ipsilateral ovary. (B) The endometrial tumors show areas of squamous differentiation. (C) Cystically dilated glands throughout the uterine horns. (D) Increased apoptosis, and numerous neutrophils infiltrating the endometrial stroma.

As with the experiments above, we confirmed that the intrauterine administration of AdLacZ remains confined to the uterus (Fig. 9) and no retrograde viral spread occurs. The only blue cells detected were in the uterus, with negative staining observed in the OSE and oviduct.

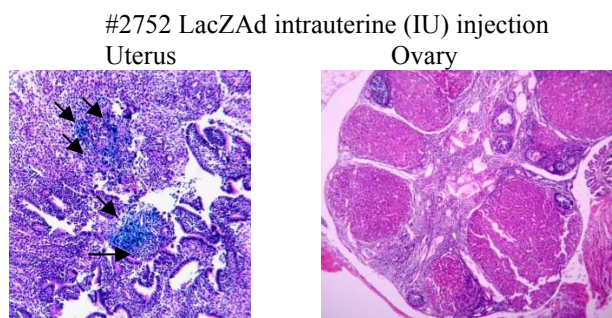


Fig. 9. HE staining of the uterus (left) and ovary (right) of a mouse injected IU with AdCre. As expected the blue staining is detected in the uterine lining (arrows, epithelial cells, left panel). No blue staining under the ovarian bursa could be detected (right panel).

5. Laser capture microdissection (LCM) of paraffin embedded tissue sections. We have recently validated the use of LCM for tumor dissection. Using this technology, we will next isolate the tumor cells and extract RNA and DNA. The DNA will be used for PCR of the Kras and Pten genes, to identify loxP recombination. The presence (absence) of recombination will support (or not) the theory on direct OSE infectivity.

Task 5 (months 9-18). Data analysis, manuscript(s) writing and submission

The characterization of MUC1KrasPten and KrasPten mice with ovarian tumors triggered by intrabursal AdCre has been completed and incorporated in a manuscript, currently under review.

We expect to have a first draft of a second manuscript, describing the morphopathogenic characteristics of lesions triggered via IB/IP/IU routes in expanded mouse cohorts, in the next 8-10 months.

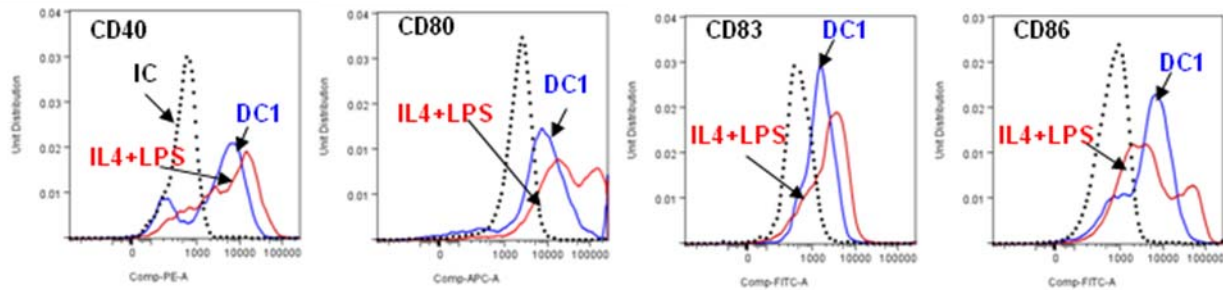
Aim 2 (Months 12-36). To profile disease heterogeneity and to identify immune biomarkers of natural and vaccine-induced immune responses in mice with either endometriosis, ovarian cancer or endometriosis progressing to ovarian cancer.

The work on this aim has been initiated.

We have already validated the in vivo potential of a dendritic cell (DC) vaccine loaded with MUC1 peptide. The dendritic cells used were matured under type 1 polarizing conditions (DC1), using a slight modification of our previously described protocol (3). This maturation cocktail (combining GM-CSF, IL-4, LPS, IFN γ and Poly I:C) is superior to other DC maturation stimuli and has been shown to enhance the DC ability to secrete high levels of IL-12 during subsequent interactions with T cells (3). The type 1 polarized, mature DC1 upregulate all emblematic co-stimulatory and maturation markers (CD40, CD80, CD86 and CD83) and display only slight phenotypic differences with classical DC (IL-4 and LPS matured) (Fig. 10A). Despite a down-regulation in IL-12 production following endotoxin-free MUC1 peptide load, compared to unloaded cells, only the DC1 (and not the LPS-matured DC) released detectable level of IL-12p70 (Fig. 10B), supporting our rationale for DC1-based vaccination.

Our DC1 studies have been performed in collaboration with Dr Pawel Kalinski, the PI's secondary mentor on the current application. The vaccination results, together with the phenotypic analysis of KrasPten and MUC1KrasPten mice with tumors are included in a manuscript (Budiu et al, under review).

A



B

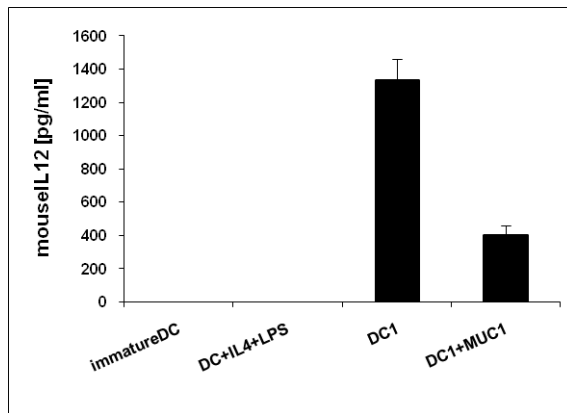


Fig. 10. (A) Phenotyping by flow cytometry of DC1 cells (blue line) and “classical” (IL4/LPS matured) DCs. (B) Detection of IL12p70 in the supernatant of DC cultures, by ELISA.

Aim 3 (Months 0-60). To validate in human specimens the disease biomarkers identified (in aim 2) in mice with endometriosis and ovarian tumors

We have secured IRB approval for our exempt studies. Accrual of specimens has been started with assistance from Dr Edwards, gynecologic oncology surgeon and primary mentor on this application. We have so far collected blood and tissue specimens from n=20 cases (10 serous, 4 clear cell, 6 endometrioid ovarian tumors).

Progress on Milestone #1: first round of publication submissions. We report that results from our experiments regarding tumorigenesis following IB injections are currently under review.

Progress on Milestone # 2: second round of publication submissions. This milestone is proposed for years 2-3.

Progress on Milestone # 3: first R01 submission. Originally planned for year 3, we report that, based on the extent and robustness of our preliminary results, we were able to submit our first R01 earlier than proposed (Feb 2011, year 1). The application has been very well reviewed, has received a high priority scores, and is currently pending the funding decision.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

- Identified that activation of Kras and PI3K pathways in the peritoneal cavity, may lead to ovarian tumors that are histomorphologically identical to those arising in mice with Kras and PI3K pathways activated in the OSE only. Further validation of these novel findings via expression profiling is currently in progress. If confirmed, our work may lead to identification of a new paradigm in ovarian tumorigenesis, one that may not require the involvement of the OSE.
- Identified for the first time that activation of the Kras and PI3K pathways in the uterine lining leads to endometrial hyperplasia, a premalignant condition, predisposing to endometrial adenocarcinoma (4). This animal model would provide unparalleled opportunities for studies on premalignant and malignant lesions of the female genital tract, with secondary ovarian involvement.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- Manuscripts- Our results from mice with tumors triggered via IB are reported in a manuscript currently under review.
- Abstracts-We have submitted during the past year one abstracts to one national meetings: (American Association for Cancer Research, AACR Annual Meeting, Orlando, FL) and one to one regional meeting (Translational Research Cancer Center Consortium, TRC3, Seven Springs, PA)
- Presentations- One invited oral presentation at TRC3 (Vlad) and two poster presentations (Budiu) at AACR and TRC3.
- Animal models- Our findings in KrasPten with lesions of the genital tract are novel and may open the door to numerous future studies in premalignancy (endometrial hyperplasia) and malignancy (peritoneal carcinomatosis, endometrial adenocarcinoma). The advent MUC1KrasPten mice with MUC1 positive genital pathology is entirely novel and will allow for identification of MUC1 roles in the above diseases and for in vivo testing of MUC1-based vaccines.
- Funding applied for based on work supported by this award-This award has supported generation of solid preliminary data, used for two major grant applications: one R01 (Feb deadline) and one ACS Investigator Award. Both grants have been well reviewed and are currently pending decision for funding.

As a reflection of the collaborative nature of the Ovarian Cancer Academy, the PI is currently collaborating with Dr Katie Terry and her mentor Dr Dan Cramer (both from Brigham and Women's) on studying the relationship between the immune status and telomere length in ovarian cancer before and after surgery. This work has been recently

incorporated in a grant proposal for the DOD Ovarian Cancer Research Program Pilot Award (August, 2011).

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

Studies completed in year one led to several novel findings regarding activation of the Kras and PI3K/Pten pathways at ovarian and extraovarian sites. If further validated (in year 2), these findings may provide support for a paradigm shift on ovarian tumor initiation.

We have also identified a new in vivo model for endometrial hyperplasia and endometrial endometrioid adenocarcinoma. This model creates unparalleled opportunities for studies in endometrial premalignancy and malignancy with or without ovarian involvement (like hormonal regulation, immune surveillance etc) and for identification of novel targets for cancer prevention and/or treatment.

The advent of the MUC1KrasPten mouse will allow for the identification of MUC1-specific roles in ovarian and endometrial tumors and in endometrial hyperplasia and for in vivo testing of MUC1-based vaccines with therapeutic or preventive potential. Human MUC1 is a well defined, valuable immune therapeutic target and our findings in these highly versatile, transgenic models have high translational potential.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science*, *Military Medicine*, etc.).

1. Dinulescu DM, et al. (2005) Role of K-ras and Pten in the development of mouse models of endometriosis and endometrioid ovarian cancer. *Nat Med* 11(1):63-70.
2. Budiu RA, et al. (2009) A conditional mouse model for human MUC1-positive endometriosis shows the presence of anti-MUC1 antibodies and Foxp3+ regulatory T cells. (Translated from eng) *Dis Model Mech* 2(11-12):593-603 (in eng).
3. Giermasz AS, et al. (2009) Type-1 polarized dendritic cells primed for high IL-12 production show enhanced activity as cancer vaccines. (Translated from Eng) *Cancer Immunol Immunother* (in Eng).
4. Kurman RJ & McConnell TG (2010) Precursors of endometrial and ovarian carcinoma. (Translated from eng) *Virchows Arch* 456(1):1-12 (in eng)

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Enclosed below is our most recent IACUC approval letter.



University of Pittsburgh

*Research Conduct and Compliance Office
Institutional Animal Care and Use Committee*

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412-383-2008
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University of Pittsburgh Protocol Number: 1104626

April 28, 2011

DOD Ovarian Cancer Academy Award

Assurance Number: A3187-01

To Whom It May Concern:

The Institutional Animal Care and Use Committee of the University of Pittsburgh has reviewed and approved on April 28, 2011 the research proposal submitted by Anda Vlad.

Titled: Disease Heterogeneity and Immune Biomarkers in Preclinical Mouse Models of Ovarian Carcinogenesis

The committee finds that the protocol meets the standards for humane animal care and use as set by the Animal Welfare Act and the NIH Guide for the Care and Use of Laboratory Animals.

Sincerely,

A handwritten signature in black ink, appearing to read "Frank J. Jenkins".

Frank J. Jenkins, Ph.D., Chair
Institutional Animal Care and Use Committee

This letter is valid until April 30, 2012.